

Remarks/Arguments:

This is a reply to the office action of June 6.

We have amended claim 1 above by reciting a first step wherein vaginal fluid samples are obtained from a population of women.

Claims 12 and 28 are similarly amended, and claim 28 has also been amended to recite that the referred group of step ii) is the one provided in the preceding step i).

In claim 33, the term "preferably" has been deleted.

Regarding the rejections related to the lack of enablement for the skilled person to reproduce the invention for all the pathologies claimed, we note that Applicant has provided at least one example for pathologies related to the adverse pregnancy outcomes and one for sexually transmitted disease and infections.

In addition, several documents representing the knowledge of the skilled man in the art have already been provided to demonstrate that several of the pathologies listed in the present invention are all linked with the presence of sialidase or prolidase activity. Accordingly, we submit that any inference of undue experimentation being necessary is overcome.

Kindly consider the following remarks regarding patentability of the invention over the prior art.

Soothill (WO 00/55354) teaches a test for sialidase activity as an indicator of BV and therefore a predictor of the likelihood of preterm birth.

An indicator of BV is not necessarily a predictor of preterm birth, for example mucinase activity, glycosulfatase, and elevated pH in vaginal fluid are all indicators of BV, but they are NOT predictive markers of preterm birth.

Particular attention is directed to Cauci et al. (American Journal Obstetric and Gynecology 2005; 192:486-96) who demonstrate that sealidase activity is *not* predictive of preterm birth (delivery at <37 week's gestation), but elevated sialidase is predictive of early preterm delivery at <35 week's and <32 week's gestation, LBW (<2500 g at birth), and very LBW (VLBW <1500 g at birth).

In addition, Soothill, as well as all the other documents, does not refer at all to a method for calculating a risk factor.

What should be understood is that Soothill discloses a diagnostic test for diseases such as BV which detects the presence of sialidase activity involving contacting a sample of a patient with a sialidase substrate (see page 3, lines 29.36).

The fact that a prior art document teaches a correlation between sialidase levels or activity with the presence of a pathology is not at all a clear and unambiguous suggestion to reach a method for calculation a risk of contracting said pathology.

In fact, a method for calculating a risk is a method based on analytical data obtained from patient's vaginal fluid but is more elaborate so that it is possible to predict if there is a risk of actually developing the pathology and, above all, how great and significant the risk is.

As stated in the introduction of the present application, several drawbacks can occur if patients are treated with conventional antibiotics for the microorganisms. Thus, the simple indication of the correlation between sialidase levels and a pathology does not

advise physicians whether the risk is really high or not compared to the possibility of causing severe drawbacks for the patients.

Moreover, it is not true that it would have been obvious for the skilled person in the art to reach the solution of the invention starting from the prior art teachings.

In fact, what is lacking in Soothill and the other documents is just the comparison between healthy people and people who contracted the pathologies in order to find out which are the critical value or thresholds actually useful for enabling physicians to decide whether to treat patients or not.

Soothill is completely silent about these matters as well as the critical values of sialidase and/or prolidase levels and of the pH value.

The fact that Soothill or other prior art discloses some sialidase or prolidase values or pH value does not suggest how to read these values together in order to understand if there is a very high, high or low risk of contracting the pathologies specified in the description of the present invention.

Accordingly, the combination of the steps as reported in claim 1 is a unique combination which can lead to the risk factor equal or higher of 5.5 OR identified only by the Applicant.

No one has previously regarded the above risk factor as actually relevant; the prior art only discloses a correlation between some sialidase and/or prolidase values and pH with certain pathologies.

Johnson (WO 00/24753) discloses that the measurement of sialidase level in vaginal samples could be used to diagnose bacterial vaginosis.

As noted above, a diagnostic marker of BV is not a predictor of adverse pregnancy outcome, i.e. clues cells, mucinase activity, glycosulfatase and elevated pH can be used to diagnose BV, but they are not predictive markers of adverse pregnancy outcome.

Further, Johnson discloses a method for the detection of sialidase using novel chromogenic substrate compounds (see page 11, lines 5-6), which method is totally different from the one disclosed and claimed in the present application.

Similarly, Lawrence (US 5,571,634) discloses elevated vaginal pH which by itself is not predictive of any adverse pregnancy outcome. A positive prolidase activity does not demonstrate a statistically significant predictive value for any adverse pregnancy outcome (see the above referenced article).

In addition, Lawrence teaches an assay performed by contacting the sample with solid-phase conjugate which is susceptible to cleavage by hydrolase and either during or subsequent thereto contacting the sample with an indicator which undergoes a detectable change upon the action of a reporter group which is a portion of the conjugate and is liberated from either partly or entirely by the action of the hydrolase (see column 4, lines 35-41).

Cauci et al. (Am J Obstet Gynecol) discovered that elevated sialidase is a marker for EARLY adverse pregnancy outcomes including extreme preterm birth at <35 week's and <32 week's gestation, LBW (<2500 g) and VLBW (<1500g), but did not find that sialidase activity is a marker for late preterm birth (at <37 week's gestation).

Similarly, Cauci et al. (J of infect Diseases) discloses the findings as above extended to elevated prolidase.

McGregor et al. (Am J Obstet Gynecol) conducted a placebo-controlled trial of the efficacy of 2% clindamycin vaginal cream (Upjohn) in pregnant women at 16-27 weeks' gestation. These authors observed that a persistent or recurrent sialidase activity 8 weeks after antibiotic treatment was a significant risk factor for LBW.

The McGregor approach implies the following steps:

- 1) Enrollment of pregnant women at >16 weeks' gestation and determination of BV status by evaluation of the following parameters:
 - a) presence of clue cells >20% on microscopic examination of saline wet mount
 - b) vaginal pH >4.5
 - c) release of amine odor after adding 10% potassium hydroxide to the saline wet preparation
 - d) evaluation of aspect of the vaginal fluid (homogenous, thin, and milky)
 - e) preparation of a dried vaginal smear
 - f) gram staining of the dried vaginal smear
 - g) evaluation of the Gram stained smear at 1000x magnification according to Nugent score (J Clin Microbiol 1991; 29:297-301.)
 - h) Determination of sialidase activity

It is very important to note that sialidase activity at enrollment has to be measured in BV-positive women, but is *not predictive of adverse pregnancy outcome*.

- 2) Treatment with 2% clindamycin cream for 7 days only to women who had BV by both clinical and microscopic criteria described above.
- 3) Determination of sialidase activity 8 weeks after completion of therapy (thus, at least 9 weeks after enrollment, meaning at > 25 weeks' gestation).

Only if sialidase activity is unchanged at 8 weeks after 7 days 2% clindamycin treatment in respect to sialidase activity at enrollment the relative risk for low birth weight (LBW) is statistically significant, whereas the risk of preterm birth at <37 weeks' gestation is not statistically significant. However, in the McGregor study the entire group of BV-positive women had no a higher risk of LBW than the non-BV group (8.6% vs 8.4%, not significant $P = 0.9$).

The McGregor results are ambiguous as the risk for LBW of BV-positive women treated with 2% clindamycin was higher than that of women treated with placebo (13.6% vs 4.4%). These results indicate that the clindamycin treatment has major negative effects and may significantly perturb vaginal fluid parameters in an unpredictable way. In other words, evaluation of sialidase activity after clindamycin treatment is a totally different parameter than sialidase activity evaluation at enrollment before/without antibiotic treatment.

The McGregor study does not teach that sialidase activity at the first antenatal visit is predictive of *any adverse pregnancy outcome* (including preterm birth, early preterm birth, low birth weight, very low birth weight, preterm labor, premature rupture of membranes, preterm premature rupture of membrane).

The McGregor study does not teach that sialidase activity without 2% clindamycin treatment is predictive of any adverse pregnancy outcome (including preterm birth, early preterm birth, low birth weight, very low birth weight, preterm labor, premature rupture of membranes, preterm premature rupture of membranes)

The McGregor study does not teach that sialidase activity irrespectively of BV diagnosis is predictive of any adverse pregnancy outcome.

The McGregor study does not teach that sialidase activity is predictive of very low birth weight (VLBW c 1500 g at birth)

The McGregor study does not teach that sialidase activity is predictive of preterm labor (PTL), premature rupture of membranes (PROM), and preterm PROM (PPROM) (all not significant results even for persistent sialidase activity after antibiotic treatment).

The McGregor study does not teach that sialidase activity is predictive of preterm birth (PTB, <37 weeks' gestation).

The McGregor study is contradictory even about data for persistent sialidase 8 weeks after antibiotic treatment and PTB; in the Abstract results are reported as non significant (15.6% vs 7.4%), whereas in Figure 5 data are reported as 15.6% vs 2% significant (P 0.03).

The McGregor study does not teach that sialidase activity is predictive of early preterm delivery at <35 weeks' gestation.

The McGregor study does not teach that sialidase activity is predictive of early preterm delivery at <32 weeks' gestation.

The McGregor study does not teach that sialidase activity is predictive of early adverse pregnancy outcomes as miscarriage.

The McGregor study does not teach that sialidase activity is predictive of stillbirth.

Briselden et al. (J of Clinical Micro) investigate whether sialidase in vaginal fluid are associated with BV and identify which organisms recovered from women with BV

contributed sialidase activity to the vaginal fluid (see page 665, right column, first sentence of Discussion).

It is evident that, once again, the document only correlates the presence of sialidase activity with BV pathology but does not suggest how to calculate the risk factor of adverse pregnancy outcome. As demonstrated above, the lack of the step of determining activity values and reporting one with the other to identify a threshold of 5.5 is fundamental to assess obviousness of the present invention.

The issue is therefore how – from the data provided by the prior art documents – the skilled person in the art could find any suggestion of performing the steps of claim 1 if the data shows a simple correlation form sialidase or prolidase activity and a pathology.

In Breselden et al., from page 665, right column to page 666, it is clearly stated that “Further studies are needed to determine whether there is a relationship between sialidase activity in vaginal fluid and prematurity, upper genital tract infection and BV”. Thus, it is evident that Briselden et al. casts doubt on the correlation of the sialidase and the pathologies, which is the opposite of what the Examiner concluded.

For the above reasons, we respectfully submit that the invention recited in claims 1 - 3, 5 - 16 and 28 - 33 is patentable over the prior art of record, and that this application is in proper form for allowance.

Respectfully submitted,

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